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NOTES

Characterization of the Rearranged *tpr-met* Oncogene Breakpoint

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We determined the nucleotide sequence of the rearranged *trp-met* genomic locus and the corresponding portions of the unrearranged *tpr* and *met* genomic fragments. The breakpoints occur at one end of a stretch of 21 A residues that follow an *Alu* repetitive sequence in the *tpr* locus and within a group of 3 A residues in the *met* proto-oncogene locus. We concluded that the fusion between the *tpr* locus on chromosome 1 and the *met* locus on chromosome 7 resulted from a recombination event.

Cellular proto-oncogenes represent a class of genes that appear to be involved in cellular transformation (29). Proto-oncogenes were originally defined as the cellular homologs of retroviral oncogenes (2), although the group has been extended by the identification of related gene family members, genes located at specific viral insertion sites or sites of chromosomal translocations, and genes with transforming activity as detected by DNA transfection assays (29). Activated cellular oncogenes have been isolated from many tumors and tumor cell lines. In some cases, activated oncogenes have been described that contain point mutations (29) or are aberrantly regulated (5). However, many activated oncogenes have been the targets of gene rearrangements resulting from chromosomal rearrangement, viral insertion, or DNA amplification. Despite the obvious importance of DNA rearrangement in the development of neoplasia, few model systems have been developed to study this problem.

Chemical carcinogens are believed to play an important role in the development of human neoplasms (3); yet, there are few examples of in vitro transformation of human cells. Thus, there has been little data directly addressing the molecular mechanisms of human carcinogenesis. The human osteosarcoma cell line HOS can be further transformed to a tumorigenic phenotype by both chemical and viral agents (22). A transforming sequence, termed *tpr-met*, was isolated from an *N*-methyl-*N'*nitro-*N*-nitrosoguanidine (MNNG)-treated tumorgenic derivative cell line, MNNG-HOS, and mapped to human chromosome 7 (7, 9). In MNNG-HOS cells (but not in parental HOS cells), *met* is joined to the *tpr* gene, normally found on chromosome 1, to form the *tpr-met* oncogene (20). To study the mechanism of this activation, we determined the nucleotide sequence surrounding the site of rearrangement.

Previous data mapped the rearrangement of the *tpr-met* gene to a 3.4-kilobase (kb) *Eco*RI fragment (pmetI) (20). To identify the breakpoint in the nucleotide sequence, we used *met* and *tpr* probes flanking the rearranged *met* oncogene I fragment described by Park et al. (20) to isolate the respective unrearranged DNA fragments from a λ phage library of human placental DNA (Fig. 1a). We screened 10^6 phage at high stringency (1, 16) by using nick-translated (23) *met* or single-stranded *tpr* probes (20). We isolated two recombinants, λtpr-1 and λ*met* C.3, and found that they contain the next downstream 5.5-kb fragment in the normal *tpr* locus and the next upstream 3.5-kb DNA fragment in the *met* proto-oncogene locus. We mapped the rearrangement to a 1.2-kb *Bgl*II fragment present in the placental λ*met* phage clone and a 2.5-kb *Eco*RI fragment present in the *tpr* clone (Fig. 1a; data not shown). We cloned these fragments and a portion of p metI (20) into M13 vectors and determined the nucleotide sequence with standard M13 sequencing primers (25) or 17-base-pair primers synthesized on an Applied Biosystems DNA synthesizer (24) (Fig. 1b).

Figure 2 displays the sequence of the normal *met* and *tpr* loci surrounding the DNA breakpoint in the *tpr-met* oncogene. By comparing the three sequences, we mapped the crossover in *tpr-met* within one of three A residues at position 525 to 527 of the *met* gene (Fig. 2A and C). We

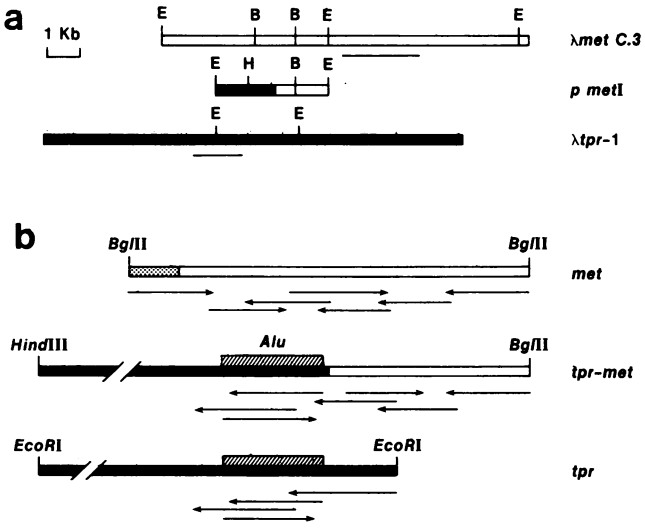

FIG. 1. λ phage map and sequencing strategy. (A) The underlined regions represent probes used to screen a human placental DNA library constructed in λL47.1. The pmetI clone was isolated from NIH 3T3 cells transformed by MNNG-HOS DNA (7). Shaded regions derive from the *tpr* locus on chromosome 1, and open regions are from the *met* gene on chromosome 7 (9, 20). Not all of the *Bgl*II sites are shown. (B) The indicated fragments were cloned into M13 mp18 and mp19 vectors, and the single-stranded DNA was sequenced (25). Abbreviations: E, *Eco*RI; B, *Bgl*II; H, *Hin*dIII.

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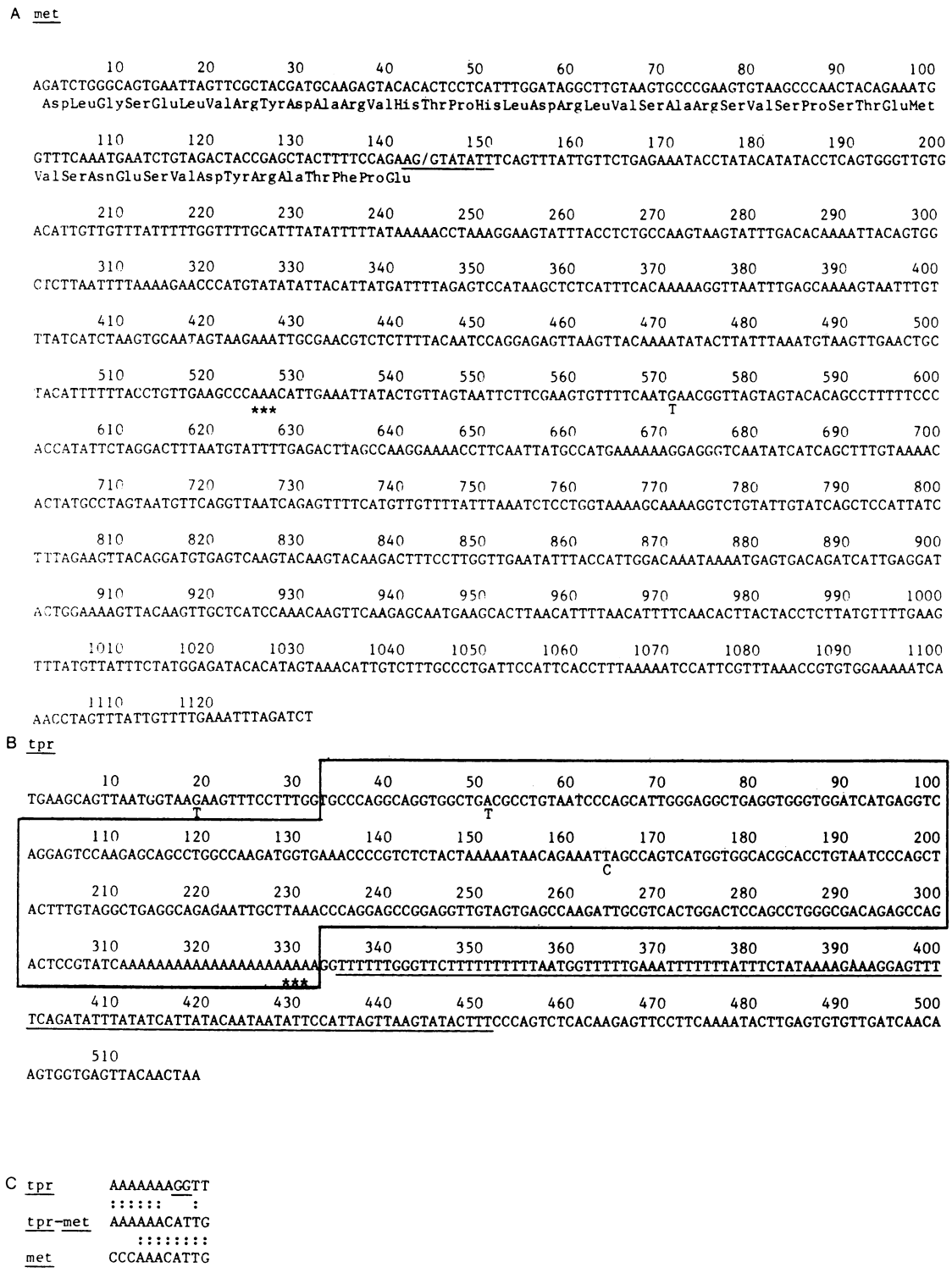

FIG. 2. Sequence of *tpr* and *met*. The nucleotide sequence of the regions of the *tpr* and *met* genes indicated in Fig. 1 are shown. Differences in the sequence between *tpr-met* and *tpr* or *met* are shown under the respective sequence. Changes were confirmed by sequencing M13 clones of the two genes with the same primer and running the reactions alongside each other on the same gel. (A) Sequence of *met*. The open reading frame in *met* homologous to a cDNA derived from the *met* proto-oncogene and the splice donor sequence at position 143 are indicated. The breakpoint occurred within one of the three A residues indicated at position 525. Nucleotide 571 (G) is a T in *tpr-met*. (B) Sequence of *tpr*. The *Alu* repeat sequence is boxed, and the rearrangement point is indicated at position 329. The three changes present in *tpr-met* are shown (positions 19, 51, and 164). The A-T-rich region following the breakpoint is underlined; no homology between this sequence and the DNA data base was detected. (C) Sequence of the breakpoint. The sequences of *tpr*, *tpr-met*, and *met* are aligned, and the 3-base-pair overlap is shown. Two G residues at the *tpr* breakpoint are underlined as possible sites of MNNG action.

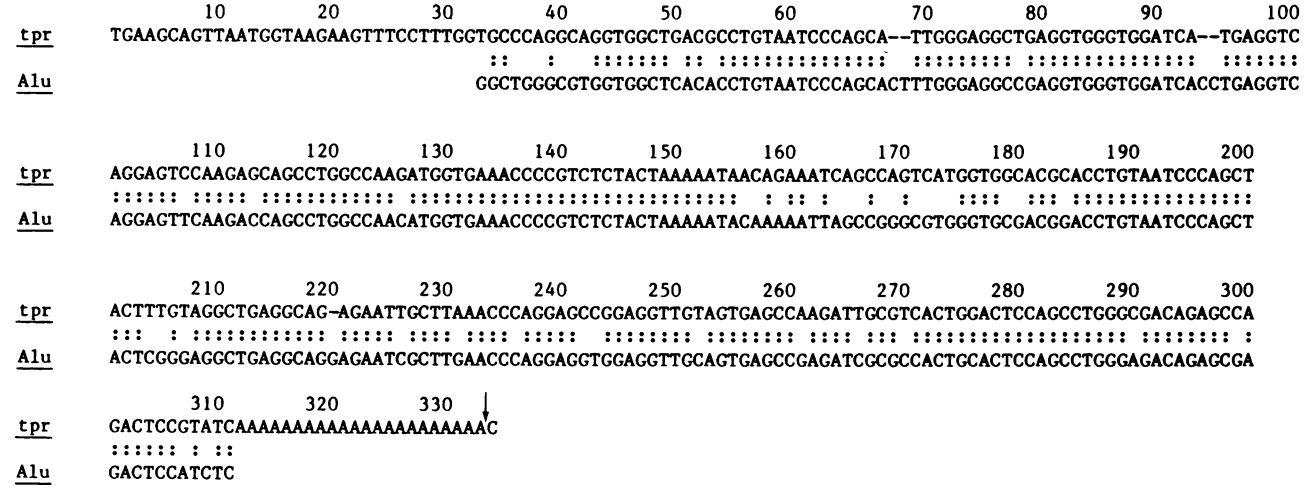

FIG. 3. Comparison of the *tpr Alu* sequence with the consensus. The *Alu* sequence from the *tpr* locus is compared with a consensus human *Alu* sequence (12).

found that the sequence of *met* and *tpr-met* are identical downstream for 600 bases from the breakpoint, except for a single-base-pair change (position 571). Upstream from the breakpoint, an open reading frame and a splice donor site are present in *met* (Fig. 2A). Sequences in the open reading frame precisely match a portion of *met* cDNA clone open reading frame (M. Park et al., manuscript in preparation). Thus, the rearrangement in the *met* locus must have occurred within an intervening sequence.

The sequence of the *tpr* gene surrounding the rearrangement is shown in Fig. 2B and C. In a region of 330 bases upstream from the breakpoint, there are only 3-base-pair changes between *tpr* and the *tpr-met* sequence. We confirmed these changes by performing sequence analysis on M13 clones of the two genes, with the same primer and running the reactions beside one another on the same gel. There is a 3-base-pair overlap between *tpr* and *met* at the breakpoint (position 525 to 527) (Fig. 2C). The rearrangement occurred at the end of 22 A residues within the *tpr* sequence (Fig. 2B). Examination of the *tpr* sequence showed that it contains an *Alu* family repeat (Fig. 2B, boxed region). Figure 3 shows an alignment of the *tpr* repeat with the consensus *Alu* sequence (12); the two sequences are 84% homologous. Other members of the *Alu* family are, on average, 90% homologous (12). *Alu* repeats are often followed by long stretches of A residues (12), and the *tpr-met* arrangement occurs at the end of the poly(A) stretch following the *tpr Alu* sequence.

Chromosomal rearrangements have been observed in many malignant cells (30). The nucleotide sequence of several other breakpoint sites have been determined and included rearranged c-*myc*, immunoglobulin heavy-chain genes (4, 8, 18), *bcl1* (28), *bcl2* (6, 27), and T cell receptor genes (10). Most of these rearrangements involve immunoglobulin joining (J) or switch region sequences and appear to involve the activity of a recombinase on specific sequence elements (4, 28). Rearrangements of this type may be restricted to cells that express this specialized recombination machinery. Therefore, one may expect that the rearrangements that occur in nonhematopoeitic cells arise by a different mechanism.

The HOS cell line contains a marker chromosome (17) which we recently identified as a derivative of chromosome 7 (M. Park, M. Gonzatti-Haces, M. Dean, D. G. Blair, J. R. Testa, D. D. Bennett, and G. F. Vande Woude, Cold Spring Harbor Symp. Quant. Biol., in press). This der (7) chromosome contains material from 7pter-q32 and 1q21-qter. As previously demonstrated, HOS cells do not have a rearranged *tpr-met* gene (20). However, it is likely that the *tpr-met* gene present in MNNG-HOS arose from the der (7) chromosome, possibly via a chromosomal inversion or deletion (data not shown).

Alu repeats have been found flanking the sites of deletions in the low-density lipoprotein receptor and globin genes (11, 15, 19). However, the *Alu* sequence at the breakpoint in *tpr* did not contribute to a homologous recombination event between two repeats as shown by the fact that no repeat is present within 4 kb upstream from the breakpoint in the *met* proto-oncogene locus (data not shown). Thus, we concluded that the *tpr-met* rearrangement resulted from a recombination event that involved limited homology. It will be interesting to see whether breakpoints detected in nonhematopoietic cells have similar structures.

It is interesting to speculate that the A-T-rich region of *tpr* downstream from the breakpoint contributed to the rearrangement. Chromosomal regions with increased lability (fragile sites) have been described previously (14) and have been proposed to play a role in chromosomal rearrangement. The A-T-rich region in *tpr* may be such a fragile site. Fragile sites have been detected by disrupting thymidine metabolism, suggesting that A-T regions may be involved (26).

The MNNG-HOS cell line containing the active *met* oncogene (22) was isolated as a morphological variant of HOS by exposing these cells to MNNG for 7 days. Although MNNG has been shown to cause point mutations, it is also clastogenic and can cause a marked increase in sister chromatid exchange (21). We cannot be sure that MNNG treatment caused the *tpr-met* rearrangement or the four point mutations we identified. The principal action of MNNG on double-stranded DNA is methylation of the N-7 position of guanine (13). A pair of G residues are located on *tpr* just downstream from the breakpoint (Fig. 2C). MNNG is also capable of methylating adenine (13); thus, MNNG-induced modification of A residue(s) at the breakpoint may have contributed to the rearrangement. Whatever its cause, the breakpoint of the *tpr-met* oncogene has a structure that is

unique among known oncogene rearrangements. These studies also suggest that HOS cells can be a useful model system for studying the effects of chemical carcinogens on human cells.

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